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MIP-1α and MCP-1 contribute to crescents and interstitial lesions in human crescentic glomerulonephritis

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MIP-1α and MCP-1 contribute to crescents and interstitial lesions in human crescentic glomerulonephritis.

Background. The precise molecular mechanisms of macrophage (Mϕ) recruitment and activation in crescentic glomerulonephritis remain to be investigated. We hypothesized that locally produced macrophage inflammatory protein (MIP)-1α and monocyte chemoattractant protein (MCP)-1 via the chemokine receptors participate in the pathophysiology of human crescentic glomerulonephritis by recruiting and activating Mϕ.

Methods. We investigated the levels of MIP-1α and MCP-1 by enzyme-linked immunosorbent assay (ELISA) in 20 healthy subjects, 20 patients with crescentic glomerulonephritis, and 41 control patients with various other renal diseases. The presence of MIP-1α, MCP-1, and the cognate chemokine receptor for MIP-1α, CCR5, in the diseased kidneys was evaluated by immunohistochemical and *in situ* hybridization analyses.

Results. MIP-1α–positive cells were mainly detected in crescentic lesions, whereas MCP-1 was mainly in the interstitium. In addition, we detected CCR5-positive cells in diseased glomeruli and interstitium. Urinary MIP-1α was detected in crescentic glomerulonephritis, even though it was below detectable levels in healthy subjects and in patients with other renal diseases without crescents. Urinary MIP-1α levels in the patients with crescentic glomerulonephritis were well correlated with the percentage of cellular crescents and the number of CD68-positive infiltrating cells and CCR5-positive cells in the glomeruli. However, urinary MCP-1 levels were well correlated with the percentage of both total crescents and fibrocellular/fibrous crescents and the number of CD68-positive infiltrating cells in the interstitium. Moreover, elevated urinary levels of both MIP-1α and MCP-1 dramatically decreased during glucocorticoid therapy-induced convalescence.

Conclusions. These observations suggest that locally produced MIP-1α may be involved in the development of cellular crescents in the acute phase via CCR5 and that MCP-1 may be involved mainly in the development of interstitial lesions in the chronic phase when fibrocellular/fibrous crescents are present, possibly through Mϕ recruitment and activation.

Key words: macrophage inflammatory protein-1α, monocyte chemoattractant protein-1, cellular crescents, rapidly progressive glomerulonephritis, CCR5.

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Crescentic glomerulonephritis is a prominent feature in rapidly progressive glomerulonephritis (RPGN) because it is associated with a comparable degree of tubulointerstitial lesions [1]. Activated intraglomerular inflammatory monocytes/macrophages (Mϕ) are presumed to be involved in the crescentic glomerulonephritis by secreting inflammatory mediators, such as cytokines and chemokines [2–5]. In addition, the interactions of infiltrating Mϕ with renal resident cells, including glomerular epithelial cells, may play a crucial role in the formation of crescents via the up-regulation of adhesion molecules and matrix formation [6]. Thus, clarifying the detailed mechanisms involved in crescentic formation would be useful for a better understanding and therapy of crescentic glomerulonephritis. A variety of factors have been implicated thus far both in the recruitment of Mϕ into the diseased glomeruli and interstitium and their activation. However, the precise molecular mechanism of the recruitment and activation of Mϕ in crescentic glomerulonephritis remains to be investigated.

A chemokine, macrophage inflammatory protein (MIP)-1α, was found to be secreted by mononuclear cells, neutrophils, and various nonleukocytic cells, including inflammatory fibroblasts, astrocytes, and mesangial cells [7–11]. Recent studies indicate that MIP-1α is involved in the pathogenesis of experimental autoimmune encephalomyelitis through Mϕ recruitment and activation [12]. Moreover, MIP-1α involvement has been demonstrated in the pathogenesis of experimental crescentic glomerulonephritis models *in vivo* [13–15]. Furthermore, recent studies revealed that the infiltration and activation of

Table 1. Patient demographics

Diagnosis	No. of patients (*male:female*)	Age (*mean years*)	Crescents %		Urinary levels *pg/mg·creatinine*		CD68 positive cells	
			Total	Cellular	MIP-1α	MCP-1	/glom	/field (× 200)
Healthy subjects	20 (10:10)	16–82 (57.1)	ND	ND	0	1.0 ± 0.1	ND	ND
Crescentic glomerulonephritis	20 (12:8)	19–82 (56.5)	74.4 ± 5.4[a]	26.2 ± 7.0[a]	4.2 ± 2.2[a]	27.3 ± 6.5[a]	8.4 ± 1.8[a]	39.8 ± 7.5[a]
ANCA-related	11 (6:5)	22–77 (61.6)	79.1 ± 6.1	15.3 ± 5.1	5.9 ± 3.9	29.3 ± 8.7	8.0 ± 4.1	26.7 ± 8.4
IgA nephropathy	6 (5:1)	19–75 (48.0)	64.0 ± 12.7	18.3 ± 10.0	2.6 ± 2.6	16.5 ± 4.0	3.4 ± 0.8	33.4 ± 13.1
Lupus nephritis	2 (1:1)	27–74 (55.5)	80.0 ± 14.1	8.0 ± 5.6	1.1 ± 1.5	60.6 ± 42.7	11.4 ± 4.8	75.0 ± 51.0
Cryoglobulinemia	1 (0:1)	82	70.0	70.0	2.4	6.3	14.0	19.5
Disease controls (without crescents)	41 (17:24)	16–72 (42.9)	0	0	0.4 ± 0.2	11.6 ± 3.5	4.9 ± 0.7	19.4 ± 3.9
IgA nephropathy	16 (10:5)	16–72 (50.0)	0	0	0.4 ± 0.4	4.5 ± 1.0	2.0 ± 0.7	12.5 ± 6.4
Lupus nephritis	18 (3:15)	22–59 (38.5)	0	0	0.5 ± 0.4	14.0 ± 5.7	4.8 ± 1.4	22.0 ± 8.5
Cryoglobulinimia	1 (1:0)	59	0	0	0	45.7	17.0	45.0
Minimal change nephrotic syndrome	6 (3:3)	17–60 (30.0)	0	0	0	1.6 ± 0.4	ND	ND
Total	81 (39:42)	16–82 (49.9)						

Abbreviations are: ANCA, anti-neutrophilic cytoplasmic antibody; ND, not done; glom, glomerulus; MIP-1α, macrophage inflammatory protein-1α; MCP-1, monocyte chemoattractant protein-1. Values are mean ± standard error of the mean.

[a] $P < 0.05$ vs. disease controls

leukocytes by MIP-1α is mediated by its cognate chemokine receptor, CCR5, which is expressed on the target cells [16, 17]. As previously reported, another chemokine, monocyte chemoattractant protein (MCP)-1, plays an important role in the pathogenesis of experimental glomerulonephritis models [2, 13, 14] and human nephritis [4, 18]. However, the distinct roles of MIP-1α and MCP-1 via chemokine receptors in the pathogenesis of human crescentic glomerulonephritis have not yet been investigated.

To determine if locally produced MIP-1α and MCP-1 participate in the pathophysiology of human crescentic glomerulonephritis by Mϕ recruitment and activation via the cognate chemokine receptor of MIP-1α, CCR5, we determined the urinary levels of both MIP-1α and MCP-1 in patients with crescentic glomerulonephritis and investigated the relationship between MCP-1, MIP-1α levels and disease activities, phases of crescentic glomerulonephritis and the chemokine receptor, CCR5.

METHODS

Patients

Twenty healthy subjects and 61 patients (39 males and 42 females; median age, 49.9 years; range, 16 to 82 years) with primary or secondary glomerular diseases were evaluated in this study (Table 1). Twenty patients had crescentic glomerulonephritis with more than 50% of the total crescents (cellular, fibrocellular, and fibrous) of all glomeruli showing RPGN clinically [19]; 41 patients with IgA nephropathy, lupus nephritis, cryoglobulinemia, or minimal change nephrotic syndrome served as disease controls without crescents. Patients with minimal change nephrotic syndrome had massive urinary protein excretion (more than 3.5 g/day). The patients in this study were chosen consecutively from August 1992 to November 1998 at Kanazawa University Hospital or its affiliate hospitals. All diagnoses were verified by renal biopsy. Urinary tract infections were ruled out in all cases by means of bacterial cultures, microscopic findings, or both, because urinary tract infection is associated with increased urinary MIP-1α and MCP-1 levels (data not shown). Whenever possible, patients did not receive immunosuppressive agents before the sample collection. All patients who were in a clinically active state were treated with glucocorticoids, including methylprednisolone pulse therapy (500 to 1000 mg/day for 3 days), during this study. Specimens from second biopsies were obtained from six patients with crescentic glomerulonephritis after glucocorticoid therapy. All renal biopsies were performed with the consent of the patients.

Pathological studies

Sixty-one kidney specimens were obtained by renal biopsy. Two observers who had no knowledge of the clinical course of the patients examined the renal tissue under light microscopy to establish the diagnosis by standard pathological methods. For patients with lupus nephritis, the World Health Organization (WHO) criteria were used for the light microscopic classification of the major forms and the active lesions of the disease [20, 21]. The percentage of the extracapillary lesions, including crescentic formation, adhesion, or both, also was counted.

Macrophage inflammatory protein-1α and monocyte chemoattractant protein-1 measurements

Spontaneously voided midstream urine catches were collected on the morning of renal biopsy. Ten milliliters of the each urine specimen were spun at 200 × *g* for five minutes at room temperature to remove cells and precipitates. Serum samples were obtained from patients at the same time. The urinary supernatants and sera were

kept frozen at −70°C until measurement. Urinary MIP-1α and MCP-1 levels were determined by an enzyme-linked immunosorbent assay (ELISA), using a specific murine monoclonal antihuman MIP-1α antibody (clone LD78) or monoclonal antihuman MCP-1 antibody (clone ME 69) as a capture and a rabbit polyclonal antibody (either anti–MIP-1α or anti–MCP-1) as the second antibody as previously described [22]. These systems are highly specific for each MIP-1α or MCP-1, because there were no cross-reactivities with other chemokines, including interleukin (IL)-8, platelet basic protein, platelet factor 4, RANTES (regulated on activation, normal T cell expression, and secreted), and growth-related gene. The recovery rate was confirmed to be more than 95% up to 3 ng/ml in these ELISA systems. The possible *in vitro* generation of MIP-1α and MCP-1 in urine samples containing cells could be excluded by immediate separation of urinary supernatants by centrifugation. All assays were performed in duplicate. The detection limits of this ELISA system were 66 pg/ml for human MIP-1α and 40 pg/ml for human MCP-1. Urinary MIP-1α and MCP-1 levels were standardized by the amount of creatinine in the urine.

Immunohistochemical studies

The presence of MIP-1α and MCP-1 proteins was demonstrated immunohistochemically on frozen tissue specimens by the indirect avidin-biotinylated alkaline phosphatase complex method with either a specific murine monoclonal antihuman MIP-1α antibody (LD78) or a specific murine monoclonal antihuman MCP-1 antibody (ME 69) as previously described [4]. Normal mouse IgG1, which had been absorbed with both human liver extracts and immunoglobulin, was used as a negative control. In addition, the absorption test was performed using a monoclonal antihuman MIP-1α or MCP-1 antibody with excess recombinant MIP-1α or MCP-1 as a negative control. Furthermore, to determine the localization of the cognate receptor of MIP-1α, CCR5, in the tissue, we demonstrated the presence of CCR5 immunohistochemically on paraffin-embedded sections by the indirect avidin-biotinylated alkaline phosphatase complex method with either a specific murine monoclonal antihuman CCR5 antibody (clone 2D7; PharMingen, San Diego, CA, USA) as described earlier here. CD68-positive cells were detected immunohistochemically on formalin-fixed, paraffin-embedded tissue sections treated by proteinase K for five to six minutes at room temperature using murine antihuman macrophage CD68 monoclonal antibody (clone KP1; Dako, Glostrup, Denmark). Glomerular chemokine receptor-positive cells and Mϕ were expressed as the number of positive cells per glomerulus, and mean interstitial chemokine receptor-positive cells and Mϕ were counted from more than 10 randomly chosen fields under high-power magnification (×200). Two independent observers also examined the immunohistochemical findings without prior knowledge of chemokine levels and the clinical courses.

In situ hybridization for MIP-1α and MCP-1

In situ hybridization procedures were the same as those previously described [23]. A partial sequence of exon 1 (5′-TCCCTCCTCACCCCCAGATTCCATTTC CCCATCCGC-3′) [24] and a partial sequence of mRNA (5′-CAGAGACTTTCATGCTGGAGGCGAGACTG CGAGCTT-3′) [25] were used for the MIP-1α or MCP-1 antisense oligonucleotide probe, respectively.

Signals were visualized by using a commercially available digoxigenin-alkaline phosphatase system (DIG Oligonucleotide Tailing kit and Nucleic Acid Detection kit; Boehringer Mannheim Biochemica, Mannheim, Germany). Negative controls were performed by replacing the antisense probe with a sense probe for MIP-1α or MCP-1, which was the exact complement of the antisense probe (5′-GCGGATGGGGAAATGGAATCTGGGG GTGAGGAGGGA-3′ or 5′-AAGCTCGCACTCTCG CCTCCAGCATGAAAGTCTCTG-3′, respectively).

Detection of antineutrophilic cytoplasmic antibody by ELISA

Antineutrophilic cytoplasmic antibody (ANCA) was detected by ELISA using microtiter plates coated with myeloperoxidase extracts for P-ANCA or proteinase-3 for C-ANCA (SRL Co., Tokyo, Japan) [26].

Statistics

Statistical significance was analyzed using the paired or unpaired Student's *t*-test, analysis of variance test, Spearman's and Pearson's correlation coefficient for the analyses of nonparametric and parametric data, and logistic regression model. $P < 0.05$ was considered to be statistically significant.

RESULTS

MIP-1α and MCP-1 levels in crescentic glomerulonephritis

Macrophage inflammatory protein-1α was detected in the urine in 11 of 20 patients with crescentic glomerulonephritis (4.2 ± 2.2 pg/mg · creatinine, mean ± SEM; Table 1 and Fig. 1). In contrast, we did not detect urinary MIP-1α in patients without crescentic formation or healthy subjects except for two patients (0.4 ± 0.2 pg/mg · creatinine, $P < 0.01$). Two patients with elevated urinary MIP-1α without crescentic glomerulonephritis had hypercellularity of Mϕ in glomeruli (19.2 per glomerulus in lupus nephritis patient and 4.8 per glomerulus in IgA nephropathy patient) and the interstitium (9.3 per field in lupus nephritis patient and 44.0 per field in IgA nephropathy patient) as revealed by the increased number

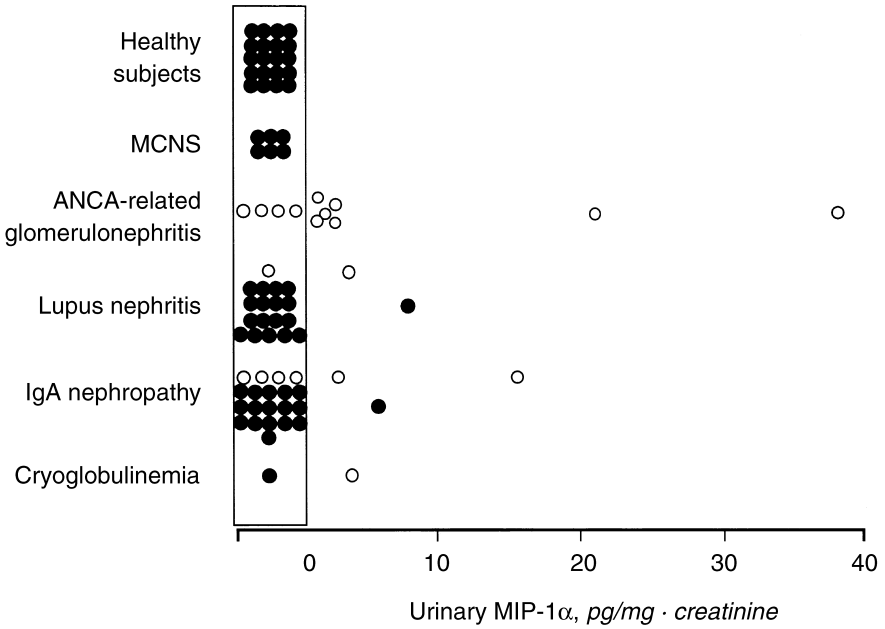


Fig. 1. Urinary macrophage inflammatory protein-1α (MIP-1α) levels in patients with various renal diseases. Symbols are: (○) patients with crescentic glomerulonephritis; (●) patients without crescentic formation. Abbreviations are: MCNS, minimal change nephrotic syndrome; ANCA, antineutrophil cytoplasmic antibody.

Table 2. Correlation with urinary levels of MIP-1α and MCP-1 and histological findings in crescentic glomerulonephritis

	Urinary MIP-1α		Urinary MCP-1	
Pathological findings	*r*	*P*	*r*	*P*
Glomerulus				
Total crescent	0.44	NS	0.48	<0.05
Cellular	0.81	<0.0001	−0.07	NS
Fibrocellular/fibrous	−0.41	NS	0.44	0.05
CD68-positive cells	0.85	<0.0001	0.30	NS
Interstitium				
CD68-positive cells	0.12	NS	0.75	<0.005
Cell infiltration	0.42	NS	0.28	NS
Fibrosis	−0.33	NS	−0.02	NS
Tubular atrophy	−0.19	NS	0.06	NS

Abbreviations are: NS, not significant; MIP-1α, macrophage inflammatory protein-1α; MCP-1, monocyte chemoattractant protein-1.

of CD68-positive cells. In addition, serum MIP-1α levels from normal subjects and all patients fell below the detection limit of ELISA. Similarly, MCP-1 was elevated in urine samples in crescentic glomerulonephritis compared with urine samples from patients with other renal diseases (27.3 ± 6.5, 11.6 ± 3.5 pg/mg · creatinine, respectively, $P < 0.05$; Table 1).

Correlation of urinary MIP-1α and MCP-1 levels and histological findings

Urinary MIP-1α correlated well with the percentage of cellular crescents ($r = 0.81$, $P < 0.0001$, $N = 20$) and the number of CD68-positive cells in glomeruli in patients with crescentic glomerulonephritis ($r = 0.85$, $P < 0.0001$, $N = 20$; Table 2). In the logistical analysis, urinary MIP-1α levels (>0.15 pg/mg · creatinine) showed a high odds ratio (OR) for the presence of cellular crescents [>10% out of all glomeruli; OR, 17.5 (CI, 0.15 to 0.34); $P = 0.0015$). In contrast, there was a significant correlation between the levels of urinary MCP-1 and the percentage of total crescents and fibrocellular and/or fibrous crescents ($r = 0.48$, $P < 0.05$, $r = 0.44$, $P = 0.05$, respectively; Table 2). In addition, urinary MCP-1 levels correlated well with the number of CD68-positive cells in the interstitium in patients with crescentic glomerulonephritis ($r = 0.75$, $P < 0.005$, $N = 20$; Table 2). However, there was no significant correlation between urinary levels of MIP-1α and MCP-1 in individual patients with crescentic glomerulonephritis.

Urinary MIP-1α levels in lupus nephritis

In 20 patients with lupus nephritis who underwent renal biopsies, the glomerular appearance was classified as pure mesangial alteration (WHO II) in 1, focal proliferative lupus nephritis (PLN, WHO Class III) in 4, diffuse proliferative lupus nephritis (DPLN, WHO Class IV) in 12, and membranous lupus nephritis (MLN, WHO V) in 3. All patients with the elevated urinary levels of MIP-1α had DPLN (2 out of 12, 16.6%), whereas urinary MIP-1α was not detected in any patients with other forms of lupus nephritis. The activity index ranged from 1 to 23 (mean 8.0 ± 1.9, $N = 20$), and two patients with DPLN with elevated urinary MIP-1α levels had a higher activity index (10 and 18, respectively).

Immunohistochemical detection of MIP-1α and MCP-1 proteins in renal tissues

To determine the local production of MIP-1α and MCP-1, renal tissue from 20 patients with crescentic glo-

merulonephritis was examined immunohistochemically for antigenic MIP-1α and MCP-1. MIP-1α–positive cells were observed in 20 patients with a similar pattern found mainly in the cellular crescents, but also in cortical tubuli, peritubular capillary endothelial cells, infiltrated mononuclear cells, and the interstitium (Fig. 2A). However, MIP-1α was faintly immunohistochemically detected in tubular epithelial cells in the kidneys from patients of other renal diseases. In addition, MCP-1–positive cells were detected in cortical tubuli, peritubular capillary endothelial cells, and infiltrated mononuclear cells in the interstitium in patients with crescentic glomerulonephritis (Fig. 2B). The staining was specific to MIP-1α and MCP-1, because neither the control isotype-matched murine IgG nor antibody absorbed with recombinant MIP-1α or MCP-1 stained positively (data not shown).

Expression of MIP-1α and MCP-1 transcripts in renal tissues

In situ hybridization detected MIP-1α mRNA signals mainly in crescentic lesions and in cortical tubuli, peritubular capillary endothelial cells, infiltrated mononuclear cells in the glomeruli and the interstitium in the same manner as the immunohistochemical analyses (Fig. 3A). In contrast, MCP-1 mRNA was detected mainly in cortical tubuli, peritubular capillary endothelial cells, and infiltrated mononuclear cells in the interstitium, but it was not detected in the glomeruli (Fig. 3 B, C). These stainings were specific, because positive staining was not detected when the tissues were hybridized with the sense oligonucleotide probe for MIP-1α or MCP-1 (data not shown).

Localization of CCR5 and correlation between CCR5 expression and urinary MIP-1α

To determine the localization of CCR5, renal tissue from 20 patients with crescentic glomerulonephritis was examined immunohistochemically. CCR5-positive cells were observed in both glomeruli and the interstitium in 20 patients in a similar pattern (Fig. 4). The number of CCR5-positive cells in both glomeruli and interstitium was significantly higher in patients with crescentic glomerulonephritis than those of the patients with other renal diseases without crescents (0.3 ± 0.1 vs. 0.04 ± 0.02 per glomerulus $P < 0.05$; 3.5 ± 1.1 vs. 1.0 ± 0.4 per field of interstitium, $P < 0.05$). In addition, urinary MIP-1α correlated well with the number of CCR5-positive cells in glomeruli in patients with crescentic glomerulonephritis ($r = 0.80$, $P < 0.001$, $N = 20$), but not correlated with those in interstitium ($r = 0.02$, $N = 20$).

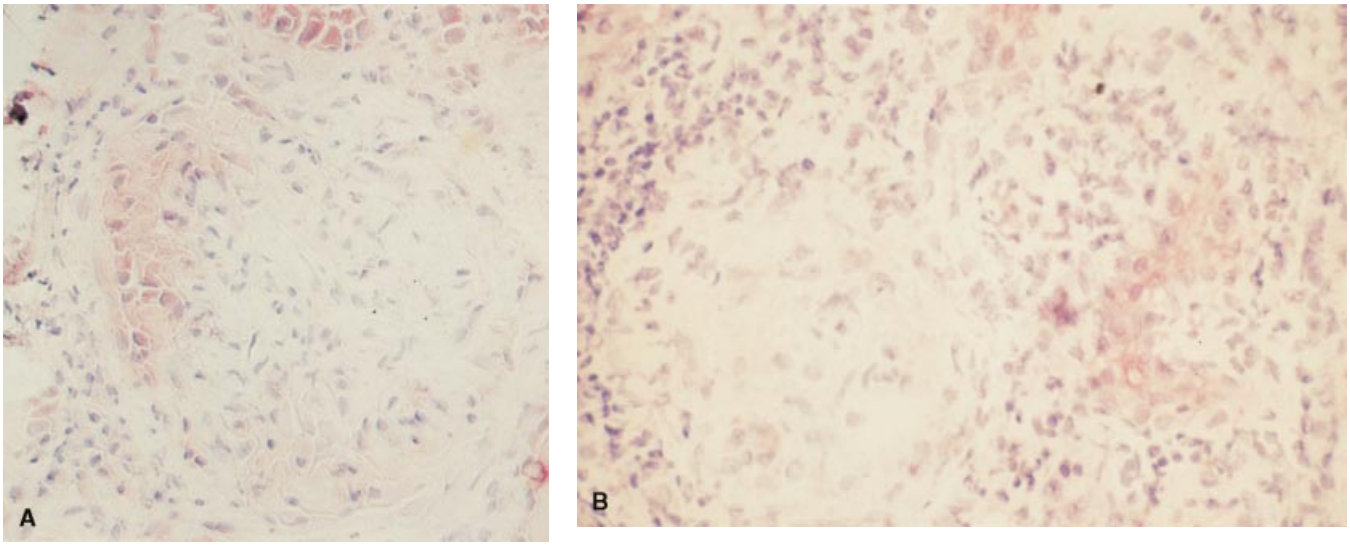
Effects of glucocorticoid therapy on urinary MIP-1α and MCP-1 levels

Twenty patients with clinically active crescentic glomerulonephritis received glucocorticoid therapy after sample collection. Elevated urinary MIP-1α and MCP-1 levels significantly decreased during convalescence induced by glucocorticoid therapy alone or with methylprednisolone pulse therapy in patients examined (MIP-1α, 4.2 ± 2.2 vs. 0.1 ± 0.1 pg/mg · creatinine, $P < 0.01$; MCP-1, 27.3 ± 6.5 vs. 12.2 ± 3.1 pg/mg · creatinine, $P < 0.05$; Fig. 5). In addition, six patients with crescentic glomerulonephritis who underwent a second biopsy had fewer cellular crescents (27.1 ± 5.3 vs. $2.1 \pm 2.1\%$, $P < 0.01$) in accordance with the decrease in MIP-1α urinary levels (4.3 ± 3.7 vs. 0.2 ± 0.1 pg/mg · creatinine, $P < 0.01$; Fig. 6).

DISCUSSION

This study demonstrated that urinary MIP-1α and MCP-1 levels are elevated in crescentic glomerulonephritis compared with patients with other renal diseases or healthy subjects. Patients with minimal change nephrotic syndrome and membraneous lupus nephritis (MLN) had undetectable levels of urinary MIP-1α or MCP-1, similar to those of healthy subjects. These results suggest that there might be no correlation between levels of urinary MIP-1α and/or MCP-1 and proteinuria caused by noninflammatory glomerular capillary lesions, such as minimal change nephrotic syndrome. Moreover, in the logistical analysis, urinary MIP-1α levels (>0.15 pg/mg · creatinine) had a high odds ratio for cellular crescents. In contrast, MCP-1 levels correlated with the degree of Mϕ infiltration in the interstitium showing fibrocellular and/or fibrous crescents. Furthermore, there was no correlation between urinary levels of MIP-1α and MCP-1 in individual patients with crescentic glomerulonephritis. Thus, we suggest that MIP-1α and MCP-1 may have distinct roles in the pathogenesis of different lesions and phases in crescentic glomerulonephritis. MIP-1α may specifically promote and escalate Mϕ infiltration into the glomeruli in the acute process leading to the formation of cellular crescents, whereas MCP-1 may participate mainly in the formation of tubulointerstitial lesions in the chronic phase, which is characterized by the formation of fibrocellular and/or fibrous crescents.

In the present study, immunohistochemical analyses confirmed that immunoreactive MIP-1α–positive cells were found mainly in the diseased glomeruli and MCP-1–positive cells mainly in the interstitium. Furthermore, *in situ* hybridization revealed that MIP-1α and MCP-1 mRNA were detected in a similar pattern. Collectively, these chemokines are produced locally in the distinct lesions of crescentic glomerulonephritis. In addition, positive cells for CCR5, the cognate receptor for MIP-1α, were immunohistochemically detected in the diseased kidneys. Regarding glomerular lesions, the percentage of cellular crescents, the number of CD68-positive Mϕ and glomerular CCR5-positive cells correlated with urinary MIP-1α levels, whereas the percentage of fibrocel-



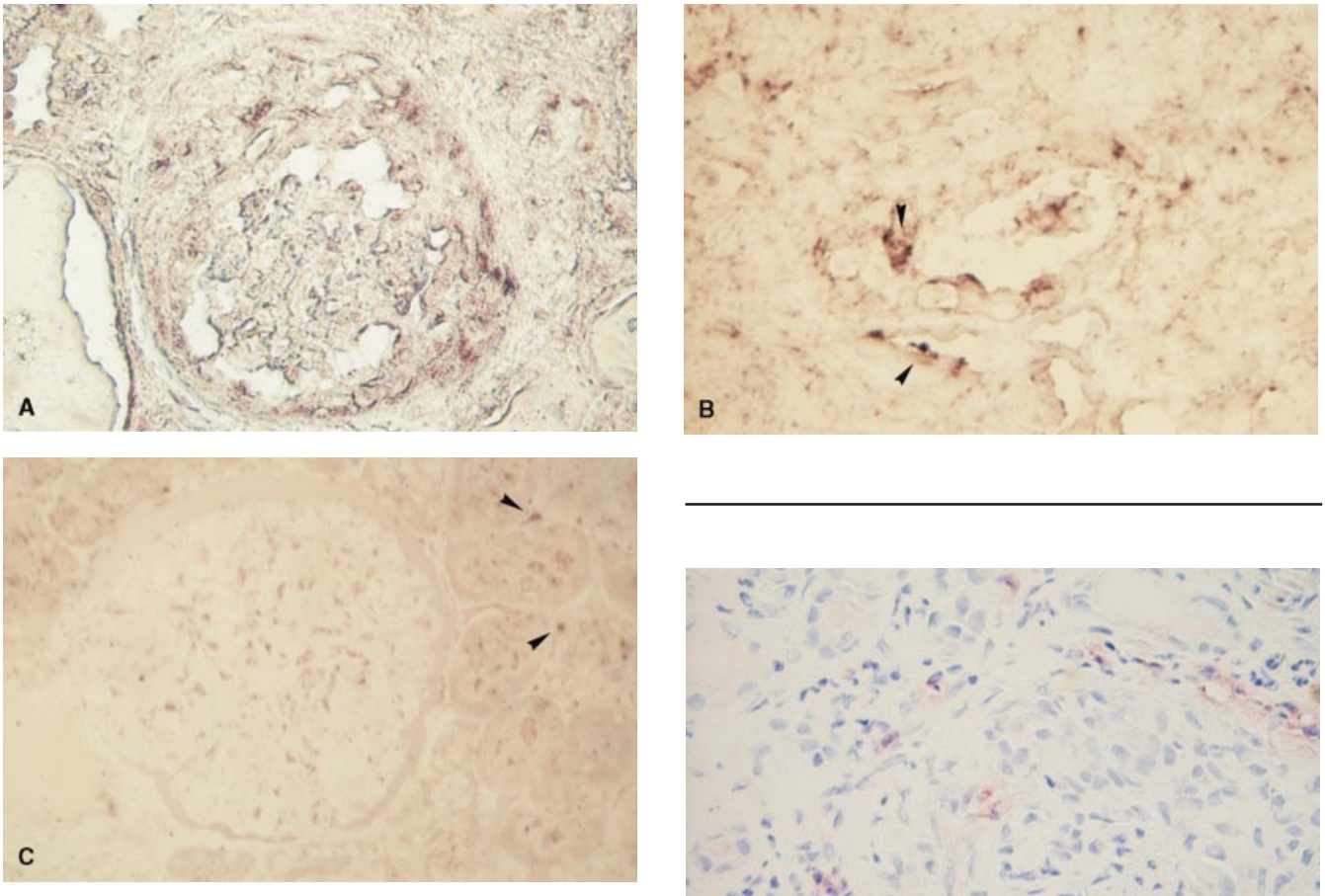


Fig. 3. *In situ* hybridization. By i*n situ* hybridization, MIP-1α mRNA signals were detected mainly in crescentic lesions and in cortical tubuli, peritubular capillary endothelial cells, infiltrated mononuclear cells in the glomeruli, and the interstitium in the same manner as the immunohistochemical analyses (*A*). In contrast, MCP-1 mRNA (arrowheads) was detected mainly in cortical tubuli, peritubular capillary endothelial cells, and infiltrated mononuclear cells in the interstitium, but was not detected in the glomeruli (*B and C*). The sections were observed under light microscopy (A–C, ×320).

Fig. 4. Detection of cognate chemokine receptor-5 (CCR5)-positive cells in the tissue. CCR5-positive cells were detected in glomeruli and interstitium in a representative patient with crescentic glomerulonephritis (×320).

Fig. 2. Immunohistochemical examination. Expression of macrophage inflammatory protein-1α (MIP-1α) or monocyte chemoattractant protein-1 (MCP-1) in renal tissues was detected using a specific monoclonal antihuman MIP-1α or MCP-1 antibody as described in the **Methods** section. The sections were observed under light microscopy (*A and B*, ×320). A representative result from one patient with ANCA-related crescentic glomerulonephritis is shown. MIP-1α–positive cells were detected mainly in crescents of glomerulus, and tubular epithelial cells and infiltrates in the interstitium (*A*). However, MCP-1–positive cells were observed mainly in the endothelial cells, tubular epithelial cells, and infiltrated cells in the interstitium (*B*).

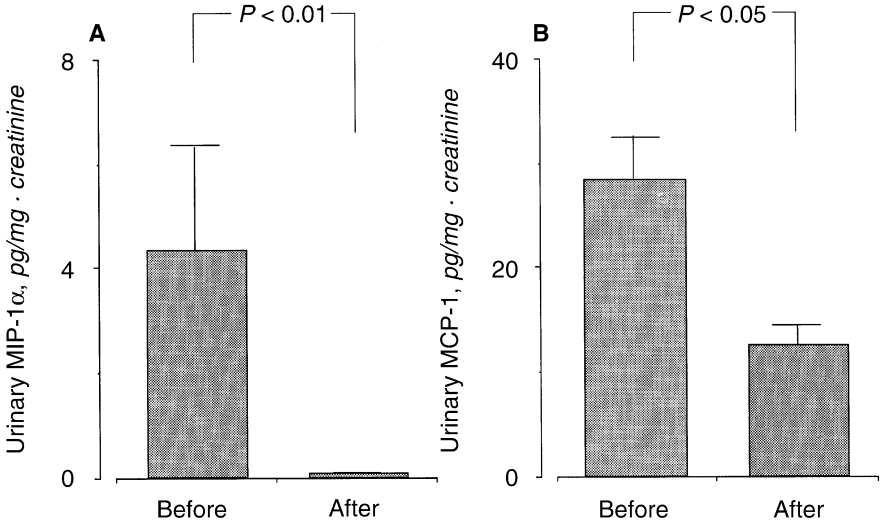


Fig. 5. Alteration of urinary MIP-1α (*A*) and MCP-1 (*B*) levels following glucocorticoid therapy in 20 patients with crescentic glomerulonephritis.

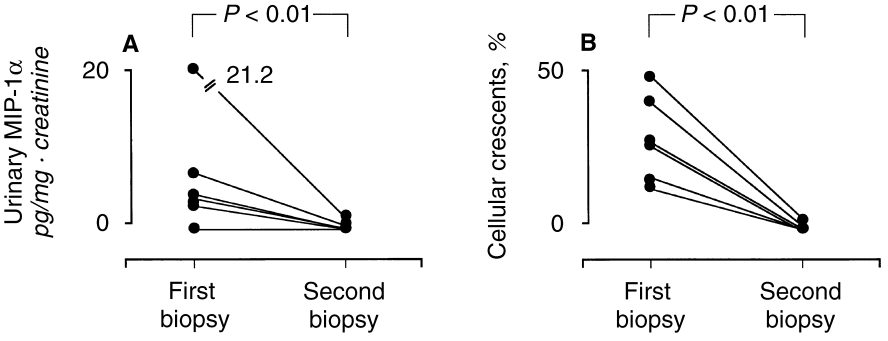


Fig. 6. Alteration of urinary MIP-1α levels (*A*) and the percentage of cellular crescents (*B*) at the first and second renal biopsies following glucocorticoid therapy, including methylprednisolone pulse therapy in six patients who underwent a second biopsy.

lular and fibrous crescents correlated with MCP-1 levels. Moreover, six patients who underwent a second biopsy had decreased percentage of cellular crescents in accordance with decreased MIP-1α levels. Thus far, CCR5 is thought to be expressed on Mϕ and T cells [16]. We determined that the cell phenotypes of the CCR5-positive cells in the kidneys are also CD3-positive T cells and Mϕ (our preliminary data). Taken together, it would be reasonable to speculate that MIP-1α may participate more specifically in the acute phases of glomerular lesion development during the formation of cellular crescents, and that the existence of chemokine receptor can confirm the functional role of MIP-1α in mediating CCR5-dependent chemotaxis in glomerular lesions in patients with crescentic glomerulonephritis. MIP-1α has been shown to induce the release of lysosomal enzymes and generate superoxide anions from Mϕ in addition to being a chemoattractant for Mϕ [9, 12]. The involvement of lysosomal enzymes, nitrous oxide (NO), and reactive oxygen intermediates (ROI) from Mϕ has been reported to play an essential role in inducing injury in renal tissue [27, 28]. Thus, it is tempting to speculate that MIP-1α may play a crucial role in the pathogenesis of cellular crescents of crescentic glomerulonephritis by recruiting CCR5-positive Mϕ and T cells, which, in turn, release lysosomal enzymes, NO, or ROI to induce crescentic formation.

The expression of MIP-1α and MCP-1 was also upregulated in the interstitium, as well as tubular epithelial cells, endothelial cells, and mononuclear infiltrates. Progressive glomerulonephritis, including crescentic glomerulonephritis, has been reported to be associated with tubulointerstitial involvement [29]. In addition, some studies

reported a strict correlation between tubular atrophy, interstitial fibrosis, the extent of interstitial infiltrates, and the renal dysfunction [5]. We previously reported that MCP-1 plays a pivotal role in the tubulointerstitial damage and promotes renal dysfunction in human lupus nephritis [4], IgA nephropathy [18], and an experimental glomerulonephritis model [2]. In support of this, cytokines diffuse from hilar areas of the glomeruli or the interstitium and affect tubular cell activation by releasing chemokines and fibrogenic factors, such as RANTES [30], osteopontin [31], and MCP-1 [15, 18, 32]. In addition, urinary MCP-1 levels correlated well with the CD68-positive cells in the interstitium, whereas MIP-1α did not. This suggests that MCP-1 may be a more important factor of the progression of tubulointerstitial lesions than MIP-1α. In addition, CCR5-positive cells were detected in interstitium as well as in glomeruli. However, urinary levels of MIP-1α did not correlate with the number of CCR5-positive cells in the interstitium, suggesting that another chemotactic factor(s) may be responsible for the induction of CCR5-positive cells in the interstitium, and that CCR5-positive cells may play an important role in the pathogenesis of both glomerular and, in part, interstitial lesions in crescentic glomerulonephritis. In contrast, the cognate receptor for MCP-1, CCR2, has been reported to mediate MCP-1–dependent chemotaxis and activation of target cells [16]. However, the mechanism of CCR2-mediated cell infiltration and activation in renal diseases, including crescentic glomerulonephritis, remains to be investigated. Therefore, we believe that the functional role of MCP-1 via CCR2-dependent chemotaxis in renal diseases should be evaluated in the future. Alternatively, massive proteinuria might induce tubular epithelial cells to activate lysosome and antigen presentation followed by the activation of helper T cells in the interstitium [33]. However, at least concerning MIP-1α and MCP-1, proteinuria itself did not induce upregulation of these chemokines because patients with minimal change nephrotic syndrome showing massive proteinuria had undetectable urinary MIP-1α and MCP-1. Taken together, once tubular epithelial cells and interstitial infiltrates have been activated by some inflammatory process, MCP-1 and, in part, MIP-1α may promote an interstitial inflammatory process as well as crescents, which is a key to the progression of crescentic glomerulonephritis through the cognate chemokine receptor-dependent chemotaxis. Collectively, urinary MIP-1α and MCP-1 levels may reflect disease activities of crescentic glomerulonephritis.

The measurement of urinary MIP-1α and MCP-1 levels may be a useful clinical tool for monitoring the distinct disease activities of crescentic glomerulonephritis. In fact, urinary levels of MIP-1α and MCP-1 fell during convalescence induced by glucocorticoids, including methylprednisolone pulse therapy. The efficacy of pulse therapy with glucocorticoids has been established for the treatment of lupus nephritis [34], IgA nephropathy [35], cryoglobulinemia [36] and RPGN [37], based on improved prognoses. Although the precise mechanism of the effects of glucocorticoids on renal injury remains to be investigated, one plausible mechanism may be inhibition of chemokines, including MCP-1 and MIP-1α. Little is known about the suppressors of MIP-1α production. Neither the glucocorticoid response element nor nuclear factor-κB (NF-κB) was identified in the 5′-flanking region of the MIP-1α gene [38]. A recent study revealed that glucocorticoids inhibit MCP-1 production at the transcriptional level in a human fibrosarcoma cell line [39], but the 5′-flanking region of MCP-1 gene contains no glucocorticoid response element up to 500 bp. It is not clear how glucocorticoids act on mesangial cells, epithelial cells, or Mϕ to inhibit MIP-1α and MCP-1 production and cause the resolution of renal diseases. Because glucocorticoids decrease the level of interferon (IFN)-γ and tumor necrosis factor (TNF)-α, the inducers of MIP-1α and MCP-1 [11], via inhibition of activity of NF-κB [40], glucocorticoids might suppress these cytokines, thereby inhibiting MIP-1α and MCP-1 production. In addition, recent studies revealed that specific neutralization of chemokines (MCP-1, MIP-1α) prevented proteinuria and renal dysfunction in experimental crescentic glomerulonephritis models [2, 15]. If this is the case, agents that specifically inhibit MIP-1α and/or MCP-1 production or block their biological functions might be replaced in the future with glucocorticoids.

In summary, our results suggest that MIP-1α and MCP-1 may be specifically involved in the pathogenesis of different phases of crescents and distinct diseased lesions via Mϕ recruitment and activation through the cognate chemokine receptor-dependent mechanism and that the measurement of urinary MIP-1α and MCP-1 levels may be useful for monitoring the different disease phases and activities of crescentic glomerulonephritis.

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